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INHIBITION OF IRON-STIMULATED CATECHOLAMINE DEGRADATION BY THE IRON-CHELATORS DETAPAC AND DESFERAL

POTENTIALLY USEFUL LABORATORY AGENTS

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Abstract—The addition of ferrous sulfate to phosphate buffer at pH 7.4 brought about a large increase in the rate of autoxidation of dopamine or norepinephrine. The iron-chelating agents diethylenetriaminepentaacetic acid (DETAPAC) and desferroxamine (Desferal) inhibited both the baseline as well as the iron-stimulated autoxidation of these catecholamines. Lesser inhibitory effects were observed with another iron-chelating agent, namely EDTA. In other experiments, DETAPAC or Desferal had little effect on the autoxidation of 6-hydroxydopamine in the absence of added ferrous sulfate, but both inhibited the ferrous sulfate-stimulated autoxidation. In contrast, both the baseline and ferrous sulfate-catalyzed rate of oxidation of 6-hydroxydopamine were greatly stimulated by EDTA addition. DETAPAC and Desferal appear to be useful experimental tools that may be added to solutions containing catecholamines to prevent their degradation.

The catecholamines are quite unstable in aqueous solutions that contain dissolved oxygen and are, for this reason, difficult to work with in the laboratory. Catecholamine stability does, however, vary over a wide range. For example, although dopamine (DA) and norepinephrine (NE) may be classified as unstable, the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) is very unstable [1]. In fact, it is generally accepted that the instability of 6-OHDA (i.e. its ease of autoxidation and formation of toxic autoxidation products) is an important determinant in its neurotoxicity [2–4].

It is well known that the autoxidation of catecholamines and of other autoxidizable compounds can be stimulated by various metal ions such as Cu^{2+} or Fe^{2+} [5–8]. The effects of iron salts are particularly relevant, in that they are common contaminants of many chemical reagents as well as of laboratory water, even water that has undergone routine distillation and/or demineralization. The present study was undertaken to determine the effects of ferrous sulfate and of several iron-chelating agents on the autoxidation of various catecholamines. The data will show that diethylenetriaminepentaacetic acid (DETAPAC) and desferroxamine (Desferal), iron-chelating agents that are relatively little-used by workers in the catecholamine field but that are now being used quite commonly by workers in the oxygen radical field [9, 10], can slow down considerably both the baseline and iron-stimulated autoxidation of catecholamines such as DA or NE.

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MATERIALS AND METHODS

The rate of autoxidation of 6-hydroxydopamine (6-OHDA · HBr) was determined by measuring the increase in absorbancy of oxidizing 6-OHDA at 490 nm on a Gilford model 300 flow-through spectrophotometer [11]. This absorbancy increase is due to the formation of quinoidal oxidation products. Experiments were run at 37° in 10 ml of a 0.05 M potassium phosphate buffer at pH 7.4. In some instances the buffer contained 10^{-4} M of an iron-chelating agent. The experiment was started by the addition of 100 μl of a 2×10^{-2} M stock 6-OHDA solution to the buffer (final 6-OHDA concentration equaled 2×10^{-4} M). A sample (100 μl) of a stock $FeSO_4$ solution was added just prior to the 6-OHDA in some experiments to give the final $FeSO_4$ concentrations indicated in the text. In other experiments, the autoxidation of dopamine (DA · HCl) or norepinephrine (NE · HCl) was determined in an identical fashion except that the absorbancy measurements were made at 480 nm.

The 6-OHDA, dopamine and norepinephrine were obtained from Regis (Morton Grove, IL) and the $FeSO_4$ was obtained from Fisher (Fairlawn, NJ). EDTA (disodium salt) and DETAPAC were obtained from Sigma (St. Louis, MO), BPS (bathophenanthroline sulfonate) was obtained from G. Frederick Smith (Columbus, OH) and Desferal from Ciba-Geigy (Summit, NJ).

RESULTS

6-Hydroxydopamine autoxidized extremely rapidly at pH 7.4 in the 0.05 M phosphate buffer (Fig. 1). DETAPAC and Desferal had no effect on this

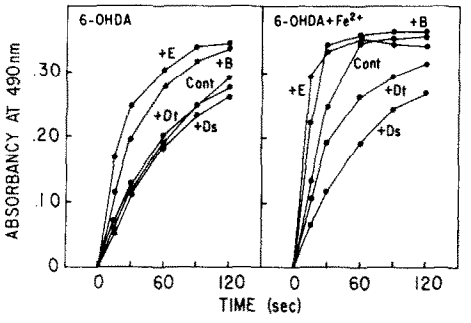

Fig. 1. Effects of various iron-chelating agents on the autoxidation of 6-OHDA in the absence (left) or presence (right) of 10^{-5} M ferrous sulfate. Experiments were done as described in the text in buffers containing no chelator (control) or 10^{-4} M Desferal (Ds), DETAPAC (Dt), BPS (B) or EDTA (E). The experiment was started by 6-OHDA addition (final 6-OHDA concentration equals 2×10^{-4} M).

baseline autoxidation while BPS and EDTA were actually stimulatory. Ferrous sulfate at 10^{-5} M stimulated the rate of 6-OHDA oxidation (compare control with and without Fe^{2+}). DETAPAC and Desferal inhibited, whereas BPS and EDTA actually appeared to enhance, the Fe^{2+} stimulated autoxidation of 6-OHDA.

The control rate of oxidation of DA (Fig. 2) was considerably slower than that of 6-OHDA (note different time scales). BPS had little effect (data not shown) whereas DETAPAC, Desferal and EDTA caused a consistent inhibition in the baseline rate of DA oxidation (Fig. 2, Desferal data not shown). Fe^{2+}, at 5×10^{-5} M, greatly stimulated the rate of autoxidation of DA; again as in the absence of added iron, BPS had little effect compared to control. In contrast, DETAPAC and Desferal markedly inhibited the Fe^{2+}-stimulated rate of DA oxidation. The inhibitory effects of EDTA on the iron-stimulated DA oxidation were considerably less pronounced than those of DETAPAC or Desferal. And in fact, from 1 hr on, the rate of DA oxidation in the presence of EDTA was similar to the control rate. Similar results were obtained on NE oxidation, except for the fact that EDTA was somewhat less effective in inhibiting the Fe^{2+}-stimulated oxidation of NE than of DA. Significant inhibition by EDTA of the rate of NE oxidation was observed only up to 0.5 hr, although at 1 hr the absorbancy in the presence of EDTA was still somewhat less than controls. In other experiments, 2.5×10^{-4} M and 5×10^{-4} M EDTA were somewhat more potent than 10^{-4} M EDTA in inhibiting the Fe^{2+}-catalyzed oxidation of DA. It should be stressed, however, that 10^{-4} DETAPAC and 10^{-4} M Desferal were both considerably more potent than either 2.5 or 5×10^{-4} M EDTA in inhibiting this Fe^{2+} stimulation.

DISCUSSION

As might have been expected, ferrous sulfate stimulated the oxidation of DA, NE and of 6-OHDA (Figs. 1 and 2). In other experiments, there was a clear-cut dose–response relationship. Higher concentrations of Fe^{2+} than those shown stimulated to a greater extent, and lesser concentrations stimulated to a lesser extent. Iron stimulation was particularly noticeable with DA or NE where the baseline rate of oxidation was slower than that of 6-OHDA. This effect of iron is not surprising in that metal salts in general are stimulants of autoxidation reactions. Moreover, iron salts are known to stimulate the autoxidation of epinephrine [6], a compound similar in structure to DA or NE and, in fact, similar in structure to 6-OHDA. In other experiments, ferric chloride (Fe^{3+}) also stimulated catecholamine oxidation, but the results were not as marked as with ferrous sulfate (Fe^{2+}).

EDTA is a metal-ion chelating agent which is often incorporated into biological systems to retard "metal ion stimulated oxidation reactions". DETAPAC, Desferal and BPS are certainly much less often utilized in biological experiments. It is probably safe to assume that most investigators would expect EDTA (or the other iron chelators) to prevent or at least inhibit whatever effect iron salts might bring about. And in fact EDTA did inhibit both the baseline as well as Fe^{2+}-stimulated autoxidation of DA and, to a lesser extent, NE (Fig. 2). DETAPAC and Desferal were better inhibitors than EDTA of both

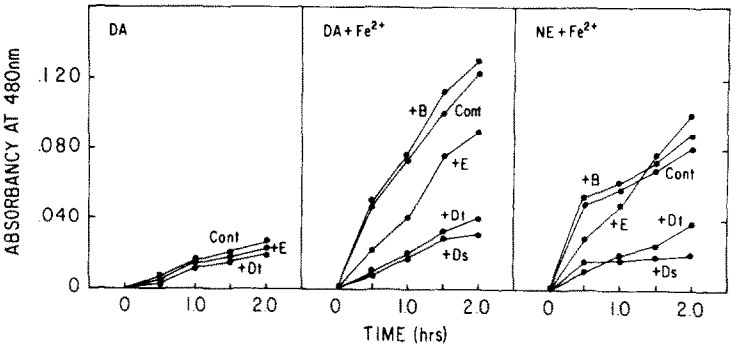

Fig. 2. Effects of iron-chelating agents on the autoxidation of DA (left) in the absence of, and on the autoxidation of DA or NE in the presence of 5×10^{-5} M ferrous sulfate (center and right respectively). Chelators are abbreviated as in the legend to Fig. 1. The experiments were started by addition of DA or NE (final concentrations equal 2×10^{-4} M).

the baseline oxidation as well as of the iron-stimulated oxidation. These iron-chelating agents had somewhat different effects on 6-OHDA oxidation than on DA or NE oxidation. DETAPAC and Desferal had little effect on the baseline, but they markedly inhibited the Fe^{2+}-catalyzed oxidation of 6-OHDA. In contrast, BPS and EDTA actually stimulated the rate of autoxidation of 6-OHDA, both in the absence and in the presence of ferrous sulfate. In other experiments, copper sulfate (Cu^{2+}) markedly stimulated the autoxidation of 6-OHDA in the absence of a chelating agent. But, in contrast to the results obtained in the present study with iron salts, Cu^{2+} had no stimulatory effect on 6-OHDA autoxidation when EDTA was present [8]. This suggests, not surprisingly, that the Fe^{2+}–EDTA complex has markedly different properties from the Cu^{2+}–EDTA complex. It should be mentioned that 10^{-4} M DETAPAC, Desferal (and EDTA) were also potent inhibitors of the $CuSO_4$ stimulated oxidation of DA. These data argue that these agents, although most often used for their chelation of iron, can effectively chelate other cations.

Now, why does EDTA augment rather than inhibit the iron-stimulated autoxidation of 6-OHDA? It is known that the autoxidation of 6-OHDA is dependent upon the superoxide radical (O_2^-). This was discovered in experiments in which superoxide dismutase, an enzyme that breaks down the superoxide radical, greatly slowed the rate of autoxidation of 6-OHDA [11]. It has been pointed out by Misra and Fridovich [6] that the presence of EDTA facilitates iron-dependent superoxide-radical dependent reactions, by virtue of its changing the electronic configuration of iron. This change results in a facilitated reaction between reduced iron and oxygen, which is a source of superoxide radicals. Thus it is not surprising that EDTA actually stimulated the rate of oxidation of 6-OHDA both in the presence and absence of added iron. If one accepts the above reasoning, it follows that DETAPAC and Desferal do not facilitate superoxide-radical dependent reactions. It should be emphasized, moreover, that even with no added iron, the phosphate buffer certainly contained a substantial concentration of iron as a trace contaminant. As pointed out above, small amounts of iron are present in phosphate salts and even in laboratory water which has been purified.

But, the autoxidation of catecholamines such as epinephrine (and dopamine) is also superoxide dependent [6]. Why then did EDTA fail to enhance the rate of autoxidation of dopamine? Misra and Fridovich have also pointed out that chelating agents such as EDTA (or perhaps also DETAPAC and Desferal) can prevent direct one electron transfer reactions between iron and a catecholamine. This would prevent formation of active catecholamine intermediates of the semiquinone type, which probably react quite rapidly with oxygen. Thus it is conceivable how, if this type of one electron transfer were important, EDTA could slow down dopamine autoxidation. These explanations to account for the differential effects of EDTA on oxidation are clearly speculative; other possible explanations are certainly viable.

EDTA is often used by investigators working with catecholamines "to retard the autoxidation of the catecholamines". The data of the present study would lead one to conclude that DETAPAC and Desferal would be preferable to EDTA in this regard in experiments with DA or with NE. This would be particularly true in experiments which take long periods of time (e.g. hours to day) to carry out, and in which catecholamine degradation may well be a limiting factor. The data of the present study also suggest that investigators working with 6-OHDA should carefully analyze their experimental systems before including EDTA or other chelating agents as "antioxidants".

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